

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-53 are in this case. Claims 3, 5, 8, 15-17, 23, 30, 34 and 39-49 were withdrawn under a restriction requirement as drawn to a non-elected invention. Claims 1, 7, 13-14, 18, 22, 24, 26, 28, 33, 38 and 53 have been objected to. Claims 1-2, 4, 6-7, 9-14, 18-22, 24-29, 31-33, 35-38 and 50-53 have been rejected. Claims 52-53 have now been cancelled. Claims 1-2, 7, 9, 13-14, 18, 19, 22, 24-26, 28, 31, 33, 35-36, 38 and 50 have now been amended.

Substitute Disclosure

The Examiner states that the disclosure is objected to because of poor quality. Please find enclosed a substitute enclosure, a request for entry and an accompanying statement specifying that the substitute disclosure does not contain additional subject matter not of record.

Drawings

The Examiner states that the drawings are objected to for reasons stated in the accompanying form PTO948. Please find enclosed replacement drawings which comply with USPTO requirements.

Claim Objections

The Examiner has objected to claims 1, 7, 13-14, 18, 22, 24, 26, 28, 33, 38 and 53 for various informalities. These claims have now been amended to correct the informalities per Examiner's suggestions.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claims 1-2, 4, 6-7, 9-14, 18-22, 24-29, 31-33, 35-38 and 50-53 under 35 U.S.C. § 112, because the specification, while being enabling for streptavidin encoding constructs with a plant signal sequence for secretion and the streptavidin processing sequences, and with and without the bacterial streptavidin signal peptide, expressed from a constitutive promoter, methods

of using them to transform plants, and plants so obtained, does not reasonably provide enablement for constructs enabling any biotin-binding protein, including derivatives of biotin and streptavidin, proteins without a secretion signal sequence or streptavidin with the processing sequences, methods of using them to transform plants, plants so obtained, or methods of plastid transformation with a construct encoding a biotin-binding protein. The Examiner's rejections are respectfully traversed. Claims 52-53 have now been cancelled rendering moot the Examiner's rejections with respect to these claims. Claims 1-2, 7, 9, 13-14, 18, 19, 22, 24-26, 28, 31, 33, 35-36, 38 and 50 have now been amended.

The present invention relates to a novel approach for controlling plant morphology via selective somatic plant tissue degeneration. Such selective somatic plant tissue degeneration enables control over crop growth and fruit yield, thereby providing growers with a tool which can be used, for example, to increase crop yield and quality.

Although the instant application provides a limited number of examples, these examples clearly illustrate that selective somatic plant tissue degeneration can be efficiently utilized to control plant morphology. In addition, these examples clearly validate the concept of the present methodology and thus provide the ordinary skilled artisan with the expectation of a reasonable degree of success necessary to make and use the claimed invention.

Applicant acknowledges that the instant specification provides results only for methods which utilize expression constructs that are capable of depleting biotin. However, the instant specification provides the ordinary skilled artisan with the guidance necessary to generate and employ expression constructs which employ additional biotin depleting polypeptides (e.g., modificants or derivatives of streptavidin) and molecules capable of depleting other essential factors such as iron, zinc and the like.

For example, the section beginning on page 36 line 13 and ending on page 39 line 6 of the published PCT application (WO 00/07427) provides numerous examples of proteins which can be used to bind and thus effectively deplete other essential factors. This section also provides evidence (cited published articles) that depletion of various essential factors using such proteins led to plant cell degeneration.

In addition to the proteins described in the instant application, numerous examples of streptavidin and avidin derivatives or modificants and other essential factor binding proteins are either commercially available or described in prior art documents.

For example, modified streptavidin can be commercially obtained from Molecular Probes (<http://www.probes.com/handbook/sections/0706.html>).

A dimeric modificant of streptavidin and methods suitable for generation of additional modificants of this protein was described by Akeshi et al. in 1997 (*Proc. Natl. Acad. Sci. USA* Vol. 94, pp. 6153-6158). In addition, Gitlin and co-workers characterized in 1990 [*Biochem. J.* (1990) 269, (527-530) (Printed in Great Britain)] the biotin-binding sites of avidin and streptavidin clearly providing one of ordinary skill in the art with the tools necessary to modify these proteins in a manner suitable for retaining biotin binding capabilities.

Aside from the references cited in the instant application, the prior art provides numerous examples of polypeptides capable of binding essential factors such as iron, zinc, thiamin, magnesium and the like (see, for example, the studies conducted by Watanabe et al. *J. of Nutr. Sci., Vitaminol.* April 1998 and October 1998 which describe Thiamin binding proteins derived from Buckwheat and sunflower seeds. Studies illustrating the effect of depleting such essential factors from plant cells have also been published see, for example, the study conducted by Van Wuytswinkel et al. (*The Plant Journal* 1999) which describes disruption in iron homeostatic in tobacco plants expressing ferritin.

Thus, although the instant specification provides a limited set of examples illustrating depletion of only one of several essential factors, as is clear from the documents cited above and the description provided in the specification, numerous examples of essential factor binding proteins as well as derivatives and modificants thereof are available to the ordinary skilled artisan. In addition, publications outlining methodology suitable for generation of additional derivatives/modificants are also available and as such, it is Applicant's strong opinion that the present invention as claimed is clearly enabled by the teachings of the instant specification.

It should be noted that although the instant application does not provide specific information describing the nucleic or amino acid sequences of such essential

factor binding proteins and modificant or derivatives thereof, one of ordinary skill in the art would be capable of (i) identifying such sequences from the relevant prior art documents and public databases (ii) generate such sequences using routine molecular procedures such as PCR and (iii) clone such sequences along with suitable regulatory elements into plants expression vectors.

The Examiner also states that the Examples in the specification teach the use of an essential secretion signal which is missing from the constructs of the broadest claims.

As is clearly illustrated in the instant specification, the use of a secretion signal, although preferred in some instances, is not essential to the depletion of essential factors. Targeting of polypeptides into a specific organelle can be replaced by expression of the polypeptides in the organelle using a specific promoter, or by transforming the organelle with the expression construct in the case of DNA containing organelles. Organelle targeted transformation methods were well known in the art prior to filing of the instant application [see for example, Planta 1996;199(2):193-201 Integration of foreign sequences into the tobacco plastome via polyethylene glycol-mediated protoplast transformation. Koop HU, Steinmuller K, Wagner H, Rossler C, Eibl C, Sacher L; Proc Natl Acad Sci U S A 1993 Feb 1;90(3):913-7 High-frequency plastid transformation in tobacco by selection for a chimeric aadA gene. Svab Z, Maliga P; and Nucleic Acids Res 1994 Sep 25;22(19):3819-24 Efficient targeting of foreign genes into the tobacco plastid genome. Zoubenko OV, Allison LA, Svab Z, Maliga P].

Plastid specific promoters are also well known in the art [for further detail please see, Plant Mol Biol 1996 Oct;32(1-2):303-14 Regulation of gene expression in plant mitochondria Binder S, Marchfelder A, Brennicke A].

Thus, the present methodology need not employ signal sequences since such sequences are optional in cases where targeted accumulation of the essential factor binding protein is not required or in cases where organelle transformation and/or organelle specific expression can be utilized in place of signal sequence mediated transport.

Although plants expressing streptavidin without the described signal sequence died at the seedling or embryo stage, it will be appreciated that use of a weaker

promoter or an inducible promoter, strategies which are well described in the instant application, can be used to produce the desired effect in instances where cytosolic expression and accumulation is employed.

Thus, in conclusion, since the instant application provides proof-of-concept and the guidance for acquiring or generating additional essential factor binding proteins as well as ample guidelines for constructing and using expression constructs, it is Applicant's strong opinion that the metes and bounds of the claimed invention are both clear to, and instantly recognizable by, the ordinary skilled artisan and that the instant specification provides sufficient guidance and incentive for the ordinary skilled artisan to make and use the invention as claimed.

In view of the above arguments, Applicant believes to have overcome the 35 U.S.C. § 112, first paragraph, rejections.

35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner has rejected claims 1-2, 4, 6-7, 9-14, 18-22, 24-29, 31-33, 35-38 and 50-53 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Claims 52-53 have now been cancelled rendering moot the Examiner's rejections with respect to these claims. Claims 1-2, 4, 6-7, 9-14, 18-22, 24-29, 31-33, 35-38 and 50 have now been amended to more clearly describe the claimed invention thereby overcoming the Examiner's rejection with respect to these claims.

With respect to claims 2 and 19, the term "fashion", now amended to --manner-- clearly describes the approach used to deplete the essential factor such that viability is retained. Thus, one can modify a level of expression to achieve such results by using an inducible or a "weak" promoter.

With respect to the term modificant, Applicant would like to direct the Examiner to, <http://complex.fiz.huji.ac.il/~mult2020/froy.html> wherein the term modificant is used to describe a variant, which as is well known in the art differs from a derivative in that unlike a derivative, it does not include a portion of the native molecule but simply includes, for example, variations in sequence or post translational modifications.

35 U.S.C. § 102/103 Rejections

The Examiner has rejected claims 1-2, 4, 6-7, 9, 12-14, 18-22, 24, 27-29, 31-33, 37-38, 50 and 52-53 under 35 U.S.C. § 102 as being anticipated by Howard et al., Baszczynski et al. or Albertson et al. The Examiner's rejections are respectfully traversed. Claims 52-53 have now been cancelled rendering moot the Examiner's rejections with respect to these claims. Claims 1-2, 7, 9, 13-14, 18, 19, 22, 24-26, 28, 31, 33, 35-36, 38 and 50 have now been amended.

The Examiner states that the prior cited describes various methods and constructs which can be used to degenerate somatic tissue and since such methods and constructs target anther tissue which is in fact somatic tissue, these prior art references anticipate the present invention.

In order to expedite prosecution in this case, Applicant has elected to amend the claims to now recite "vegetative tissue" instead of somatic tissue. As is well known in the art, vegetative tissue constitutes non-floral tissue and as such, this limitation which does not encompass anther tissue distinguishes the present invention as claimed from the teachings of the prior art cited by the Examiner. Support for this claim amendment can be found throughout the specification (see for example, page 25 lines 10-13).

Thus, it is Applicant's strong opinion that the prior art cited by the Examiner does not anticipate or render obvious the invention as now claimed.

In addition, Applicant is also of the opinion that the prior art cited by the Examiner does not in combination with any other prior art references (e.g., Marianni et al. or Maliga et al.) render obvious the present invention as now claimed.

Applicant is of the opinion that one of ordinary skill in the art would not be motivated to combine the teachings of Baszczynski et al. and Marianni et al. or Maliga et al. to make the present invention as now claimed.

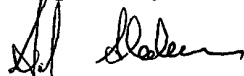
The prior art cited by the Examiner in the 35 U.S.C. §102 rejection describes methods of inducing male sterility via degeneration of floral tissue. Marianni et al. and Maliga et al. describe degeneration of somatic tissue using an enzyme such as a protease or DNase which is toxic to plant cells and the effects of which cannot be readily reversed. Although both Marianni et al. and Maliga et al. describe methods which in fact lead to degeneration of somatic tissue, such methods are also directed at

generating plants which are sterile (cytoplasmic sterility). Since neither Marianni et al. nor Maliga et al. or for that matter Baszczynski et al. describe or suggest that vegetative cell/tissue degeneration can be utilized for anything but generation of sterile plants, and since these references do not expound on the merits of reversible tissue degeneration, it is Applicants strong opinion that the present method as now claimed is not rendered obvious by the combined teachings of Baszczynski et al. and Marianni et al. or Baszczynski et al. and Maliga et al.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned **"Version with Markings to Show Changes Made."**

In view of the above amendments and remarks it is respectfully submitted that claims 1-2, 4, 6-7, 9-14, 18-22, 24-29, 31-33, 35-38 and 50-51 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,



Sol Sheinbein

Registration No. 25,457

Date: May 25, 2003.

Encl.:

Three months extension fee

Substitute Disclosure along with statement

Accompanying statement specifying that the substitute disclosure does not contain additional subject matter not of record

Substitute Drawings

Articles by:

Binder et al.

Gitlin et al.

Koop et al.

Rapala-Kozik et al

Sano et al

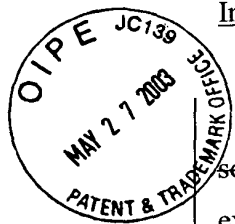
Van Wuytswinkel et al.

Watanabe et al.

Zoubenko et al.

VERSION WITH MARKING TO SHOW CHANGES MADE

In the Claims:



1. (Amended) A method of effecting degeneration of a ~~somatic~~somatievegetative plant tissue of a plant, the method comprising the step of expressing in cells of the ~~somatic~~somatievegetative plant tissue a heterologous protein capable of binding a plant essential factor, wherein said step of expressing said heterologous protein is effected in a ~~fashion~~manner; so as to lead to depletion of said essential factor such that plant viability is maintained, while at the same time, degeneration of the ~~somatic~~somatievegetative plant tissue is effected.

2. (Amended) The method of claim 1, wherein said ~~fashion~~manner is selected according to at least one criterion selected from the group consisting of:

- (i) a level of expression of said heterologous protein;
- (ii) a distribution of said heterologous protein in said plant tissue;
- (iii) binding activity of said heterologous protein toward said plant essential factor;
- (iv) abundance and distribution of said plant essential factor in said cells; and
- (v) a level of said factor externally provided to the ~~somatic~~somatievegetative plant tissue.

7. (Amended) The transgenic plant of claim 46, wherein said heterologous protein is selected from the group consisting of avidin, streptavidin ~~and~~, biotin binding derivatives and modificants thereof.

9. (Amended) The method of claim 1, wherein said heterologous protein is expressed within the cytoplasm of said cells of the ~~somatic~~somatievegetative plant tissue so as to lead to said depletion of said essential factor present within said cytoplasm, such that said plant viability is maintained, while at the same time, said degeneration of the ~~somatic~~somatievegetative plant tissue is effected.

10. (Amended) The method of claim 1, wherein said heterologous

protein is expressed within a DNA containing organelle of said cells of the ~~somatic~~vegetative plant tissue so as to lead to said depletion of said essential factor present within said DNA containing organelle, such that said plant viability is maintained, while at the same time, said degeneration of the ~~somatic~~vegetative plant tissue is effected.

11. (Amended) The method of claim 1, wherein said heterologous protein includes a leader peptide capable of self targeting into a DNA containing organelle, such that when said heterologous protein is expressed within the cytoplasm of said cells of the ~~somatic~~vegetative plant tissue said leader peptide directs said heterologous protein into said DNA containing organelle, so as to lead to said depletion of said essential factor present within said DNA containing organelle such that said plant viability is maintained, while at the same time, said degeneration of the ~~somatic~~vegetative plant tissue is effected.

13. (Amended) The method of claim 1, wherein said degeneration of plant ~~somatic~~vegetative tissue is effected for controlling-a morphology of the plant.

14. (Amended) The method of claim 1, wherein said degeneration of plant ~~somatic~~vegetative tissue is effected for controlling-a development of the plant.

18. (Amended) A transgenic plant expressing a heterologous protein capable of binding a plant essential factor, wherein expressing said heterologous protein is effected in a ~~fashion~~manner so as to lead to a depletion of said essential factor such that plant viability is maintained, while at the same time, degeneration of ~~somatic~~vegetative plant tissue of the transgenic plant is effected.

19. (Amended) The transgenic plant of claim 18, wherein said ~~fashion~~manner is selected according to at least one criterion selected from the group consisting of:

- (i) a level of expression of said heterologous protein;
- (ii) a distribution of said heterologous protein in said plant tissue;
- (iii) binding activity of said heterologous protein toward said essential factor;
- (iv) abundance and distribution of said essential factor in said cells; and
- (v) a level of said factor externally provided to said ~~somatic~~vegetative plant tissue.

22. (Amended) The transgenic plant of claim 18, wherein said heterologous protein is selected from the group consisting of avidin, streptavidin and, biotin binding derivatives and modificants thereof.

24. (Amended) The transgenic plant of claim 18, wherein said heterologous protein is expressed within a the cytoplasm of ~~somatic~~vegetative cells of the transgenic plant, so as to lead to said depletion of said essential factor present within said cytoplasm, such that said plant viability is maintained, while at the same time, said degeneration of said ~~somatic~~vegetative cells is effected.

25. (Amended) The transgenic plant of claim 18, wherein said heterologous protein is expressed within a DNA containing organelle of ~~somatic~~vegetative cells of the transgenic plant, so as to lead to said depletion of said essential factor present within said DNA containing organelle, such that said plant viability is maintained, while at the same time, said degeneration of said ~~somatic~~vegetative cells is effected.

26. (Amended) The transgenic plant of claim 18, wherein said heterologous protein is targeted into a DNA containing organelle of ~~somatic~~vegetative cells of the transgenic plant following expression thereof within a the cytoplasm of said ~~somatic~~vegetative cells, so as to lead to said depletion of said essential factor present within said DNA containing organelle, such that said plant viability is maintained, while at the same time, said degeneration of said ~~somatic~~vegetative cells is effected.

28.____ (Amended) A transgenic plant comprising ~~somatievegetative~~ plant cells—being transformed with an expression cassette including a first polynucleotide segment under a transcriptional control of a plant promoter, wherein said first polynucleotide segment ~~eneoding—~~encodes a heterologous protein which binds a sufficient amount of a plant essential factor to thereby cause degeneration of a ~~somatievegetative~~ plant tissue, while at the same time, maintain plant viability.

31.____ (Amended) The transgenic plant of claim 28, wherein said promoter is a plant derived promoter ~~and-or~~ a plant virus derived promoter.

33.____ (Amended) The transgenic plant of claim 28, wherein said heterologous protein is selected from the group consisting of avidin, streptavidin and, biotin binding derivatives and modificants thereof.

35.____ (Amended) The transgenic plant of claim 28, wherein said expression cassette transforms a genome of a DNA containing organelle of said ~~somatievegetative~~ plant cells such that said heterologous protein is expressed within said DNA containing organelle, so as to lead to said depletion of said essential factor present within said DNA containing organelle, such that degeneration of said ~~somatievegetative~~ plant tissue is effected.

36.____ (Amended) The transgenic plant of claim 28, wherein said expression cassette further includes a second polynucleotide segment coding for a leader peptide capable of self targeting into a DNA containing organelle, said second polynucleotide segment being in frame to said first polynucleotide segment, such that when said expression cassette is expressed within a cytoplasm of said ~~somatievegetative~~ plant cells, said leader peptide directs said heterologous protein into said DNA containing organelle, so as to lead to said depletion of said essential factor present within said DNA containing organelle, such that said degeneration of said ~~somatievegetative~~ plant tissue is effected.

38. (Amended) The transgenic plant of claim 28, wherein said heterologous protein is expressed within ~~a~~ the cytoplasm of said ~~somatic~~ somatic vegetative plant cells, so as to lead to said depletion of said essential factor present within said cytoplasm, such that said degeneration of said ~~somatic~~ somatic vegetative plant tissue is effected.

50. (Amended) A plant comprising ~~somatic~~ somatic vegetative tissue expressing a heterologous protein being bound to a plant essential factor, such that unbound and active form of said plant essential factor is depleted from said ~~somatic~~ somatic vegetative plant tissue, thereby effecting degeneration of said ~~somatic~~ somatic vegetative plant tissue.

In the Specification:

Paragraph beginning at page 9, line 13, has been amended as follows:

-- FIG. 5 demonstrates regeneration of degenerated somatic plant tissue in a T0 plant expressing the sps streptavidin cassette by external addition of biotin. Transformed plant cells were grown in culture in the presence of biotin until T0 plants were developed. The T0 plants were transferred to soil without further biotin supplementation. Within a month (~~0d~~ Fig. 5a) severe plant somatic tissue degeneration was evident. ~~Top~~ photographs represent Fig. 5a represents a plant having non vital young chlorotic leaves (~~0d~~). Application of biotin solution restored normal leaf development as can be seen 10 days after application (~~10d; central photographs~~ Fig. 5b), or 20 days after initial application (~~20d; lower photographs~~ Fig. 5c).--

Paragraph beginning at page 47, line 7, has been amended as follows:

-- Tomato plants were transformed with the sps construct, and 34 plantlets that were found to contain the transgene were transferred to the greenhouse. Twenty-four plants suffered of severe stem degeneration at the stage of four true leaves, and died. During the development of the remaining transgenic tomato plants, relatively minor stem and leaves degeneration could be observed in four plants, however, to a different level and time of appearance in the development of these plants (~~Figure 5, 0d~~ Fig. 5a and Table 4).--

Paragraph beginning at page 48, line 1, has been amended as follows:

-- Spraying of 6 mg/liter biotin on the affected area, stopped the degeneration process and plants' growth was restored (~~Figure 5, 10d and 20d~~ Figures 5b and 5c). Without spraying of biotin, the plants were completely degenerated, indicating that the phenotype observed was related to the streptavidin expression. The morphology and development of leaves of 6 weeks old plants obtained from non transgenic plant and transgenic tomato plants expressing the sps streptavidin construct and treated daily with biotin were also examined. Figure 7 depicts the results of this study. Transgenic plants expressing the sps streptavidin construct and treated once (after 3 weeks) with biotin and transgenic plants expressing the sps streptavidin construct and not treated with biotin showed severe morphological changes resultant from tissue degeneration. This degeneration was to a lesser degree in the treated plants. Non transgenic plants both biotin treated and untreated appeared normal. --